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Please send me:

1) Hawkins et al.
"Adapting antibodies for clinical use-monoclonal antibody engineering technology: a review"
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2) Obrist etal
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FcαRI (CD89) AS A NOVEL CYTOTOXIC TRIGGER MOLECULE FOR BISPECIFIC ANTIBODIES. B. Stockmeyer*, T. Valerius*, R. Repp*, Y. Deo *†, A. van Spiek *#, J. R. Kalden*, J. G. J. van de Winkel *#†, and M. Gramatzki *. Dept. of Medicine III, University of Erlangen-Nürnberg, Germany, † Medarex, Inc., Annandale, NJ and Medarex Europe, B.V., Utrecht, The Netherlands, # Dept. of Immunology, University Hospital Utrecht, The Netherlands

Promising results from clinical trials with unconjugated antibodies stimulated renewed interest in immune effector mechanisms of monoclonal antibodies. We investigated the potential of IgA as antibody isotype for cell- or complement-mediated tumor cell lysis, and assessed the potential of its myeloid Fc receptor - FcαRI (CD89) - as trigger molecule for bispecific antibody-mediated immmunotherapy. Comparing hapten antibodies of human IgA2 with IgG1 or IgG3 isotypes, we found all three to mediate effective killing of sensitized target cells in whole blood assays. Analysis of effector mechanisms revealed IgG-mediated lysis to be predominantly complement-dependent, whereas IgA-dependent killing was primarily effector cell mediated. A comparison of effector cell populations in ADCC demonstrated neutrophils to be most important for IgA-dependent tumor cell killing. This involved FcαRI as a cytotoxic trigger molecule - as shown with Fc receptor blocking antibodies. Reverse ADCC experiments against target cells sensitized with Fc receptor antibodies or in assays with FcαRI directed bispecific antibodies confimed FcαRI as effective triggermolecule in PMN-mediated lysis. During G-CSF therapy, [FcαRI x HER-2/neu] bispecific antibodies induced enhanced killing of HER-2/neu positive SK-BR-3 breast cancer cells in whole blood assays. This enhanced cytotoxicity was paralleled by increased PMN counts, which lead to higher effector to target cell ratios in G-CSF primed blood. Furthermore, bispecific antibbodies - directed to FcaRI and Candida albicans - mediated effective phagocytosis of fungi by neutrophils In summary, these results identify IgA as an effective antibody isotype for immunotherapy, working primarily via FcαRI. They suggest FcαRI- directed bispecific antibodies and G-CSF to be an attractive combination for malignant or infectious diseases.

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CHARACTERIZATION OF CHEMOKINE-ANTIBODY FUSION PROTEINS FOR CANCER IMMUNOTHERAPY. Pia-Maria Challita*, Camille N. Abboud, Karen E. Rosell*, Joseph D. Rosenblatt*.

The successful eradication of cancer cells during minimal residual disease may require the targeting of widely scattered tumor deposits, that may be sheltered from T cell or NK cell immune recognition by various adaptation pathways (downewgulation of Class I or II expression) and effector cell inhibitors such as TGF beta and interleukin-10 secretion. We describe here the construction of antibody fusion molecules with variable domains directed against her2*neu*, linked to sequences encoding the chemokine RANTES. The latter was chosen because of its wide spectrum of biologic activity in recruiting T cells, NK cells monocytes and dendritic cells. Moreover, RANTES has been shown to promote anti-tumor immune responses and abolish tumor establishment in syngeneic murine tumor models. RANTES cDNA was amplified by PCR and cloned at the 5'-end of human her2*neu* heavy chain *via* $(Gly_4\text{-}Ser)_3$ flexible linker. The expression vector of the light chain and heavy chain anti-her2*neu* fusion protein were transfected into Sp2/0 myeloma cells, and recombinant proteins purified. Fusion of RANTES to the antibody sequences did not alter antigen binding properties to her2*neu* transfectants and SKBR3 breast cancer cell line. The chemokine avtivity of RANTES in these engineered fusion products was demonstrated using several assays: 1) F-actin polymerization of THP-1 cells treated with dBcAMP (1.5 fold increase), 2) transwell and transendothelial migration of primary CD34+ cells and T lymphocytes. Antibody fusion proteins may overcome limitations of standard antibody therapy and represents a novel and promising approach to the problem of minimal residual disease.

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INFLUENCE OF ALLOGENEIC TH-1 AND TH-2 TYPE CD4+ T CELLS ON GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-LEUKEMIA (GVL) EFFECTS IN MICE M. Zeis, L. Uharek, B. Glass, P. Dreger, J. Steinmann*, N. Schmitz. Dept. of Internal Medicine II and Institute for Immunology*, University of Kiel, Germany

The administration of donor lymphocytes for the prophylaxis or treatment of leukemia relapse after allogeneic BMT is hampered by the high incidence of severe graft-vs-host-disease (GVHD). In the present study we determined the potential of Th1 and Th2 type $CD4^+$ T cells in mediating GVHD and GVL effects in a fully allogeneic murine transplant model. *Methods:* BALB/C (H-2^d) mice were given a dose of A20 (H-2^d, B cell leukemia) cells 2 days prior to lethal total body irradiation (TBI) and transplantation allogeneic (C57BL/6, H-2^b) anti-Thy1.2 (CD90) depleted bone marrow cells. For the generation of Th1 and Th2 type T cells donors were injected (i.p.) for 5 days with rhIL-2 (50,000 U/ml) or rhIL-2 (25,000 U/ml) + rmIL-4 (500ng), respectively. Graded numbers of either Th1 or Th2 primed $CD4^+$ donor type T cells (10^6 or 10^7) were given 2 h after BMT. *Results:* Injection of A20 leukemia into normal BALB/C recipients led to death after a median of 21 days. A lethal dose of TBI followed by allogeneic Thy1.2 depleted BMT resulted in a modest antileukemic effect with 20% of mice achieving a longlasting freedom from relapse (FFR). Whereas the infusion of 10^7 Th1 $CD4^+$ T cells given at time of BMT led to death of all mice within 50 days due to fatal acute GVHD, infusion of 10^6 Th1 type T cells resulted in modest GVHD and prolonged the survival of leukemia bearing mice significantly (FFR 50%). However, the administration of 10^6 or 10^7 Th2 type $CD4^+$ donor T cells after BMT resulted in an even stronger GVL effect (FFR 70% and 85%, respectively). Although clinical signs of GVHD were observed in mice receiving Th2 cells their incidence and severity was less frequent as compared to Th1 donor T cells. *Conclusions:* Our results demonstrate that Th2 type $CD4^+$ T cells are superior to Th1 type T cells in their potential to eradicate residual leukemia cells after BMT without mediating severe acute GVHD.

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HLA-DP EXPRESSION AND SENSITIVITY TO LYSIS BY AN HLA-DP SPECIFIC T CELL CLONE OF FRESH LEUKEMIC BLASTS. C. Ibisch*, G. Gallot*, R. Vivien*, M-M. Hallet*, N. Milpied, R. Garand, H. Vié*. Institut National de la Santé et de la Recherche Médicale, INSERM U463, and Service d'Hématologie CHU Nantes, France.

After allogenic BMT it is now clear that the anti-leukemic effect is not entirely due to the myeloablative chemotherapy and radiotherapy of the preparative regimen. Additive beneficial effect, referred to as graft versus leukemia effect (GVL), was evidenced mainly by clinical studies demonstrating that patients who experienced GVHD had fewer relapses than patients without GVHD. These two aspects of post BMT alloreactivity (GVH and GVL) are both mediated by donor T lymphocytes, which led several groups to consider different adoptive T cells transfer strategies to induce a GVL effect. In line with such strategies, we recently proposed to use cytotoxic HLA-DP specific T cell clones to induce and control a GVH-GVL reaction. In brief, the rational is as follows : i) among recipients of unrelated HLA-A, -B, -DR identical bone marrow transplantation, 70% are DP mismatched ii) HLA-DP disparity is not recommended as an exclusion criterion for donor selection in unrelated marrow transplantation iii) Nevertheless, HLA-DP antigens are clearly involved in post-BMT alloreactivity. Consequently, a possible situation to produce a GVH-GVL effect while sparing the new hematopoiesis is a T-cell-depleted-allo-BMT in which a T cell clone, transfected with a suicide gene to allow an in vivo control of its proliferation, would target an HLA-DP mismatch in the GVHD direction. This kind of transplantation would allow a phase I clinical trial in an otherwise immunologically " classic " situation. For this strategy to be successful, HLA-DP antigens should be present on leukemic cells and recognized by HLA-DP specific T cell clone with subsequent cytotoxicity. In line with this approach, the present study was initiated to analyse HLA-DP expression on leukemic cells as well as their sensitivity to direct CTL lysis. Firstly, differential expression of HLA-DR, -DQ and -DP was tested by fluorescence using monoclonal antibodies on a panel of 43 acute myeloid leukemias (AML), 39 acute lymphoblastic leukemias (ALL), 37 chronic lymphocytic leukemias of B-cell origin (B-CLL), 11 chronic myelogenous leukemias acutely transformed (CML-AT) and 16 lymphomas. Results demonstrated that HLA-DR, -DQ and -DP was detectable on AML (80%, 50%, and 63% respectively), ALL (89%, 57%, and 85%) CLL-B (100%, 87%, and 97%), CML-TA (64%, 36%, and 64%) and lymphomas (83%, 32%, 75%). Thus, the vast majority of leukemic cells express HLA-DP antigens. Next, a panel of CLL-B, AML and ALL was genotyped for HLA-DP and used as target cells in a cytotoxic assay to test their sensitivity to lysis by a CD4+ cytotoxic HLA-DPB1*0401 specific T cell clone. Specific recognition of leukemic blasts could be demonstrated for all B-CLL, for 8 out of 12 AML and for 13 out of 15 ALL. These datas show that most leukemic blasts are accessible to direct lysis by allogeneic HLA-DP specific T cells.